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The Radegen Biosynthetics (TM) Core Product line. Each item in the first bulleted category starts with a name beginning with the greek character theta (θ) followed by a descriptive name interrupted by period characters instead of spaces. This label is the official product name for each product line followed by a shorthand term used after introducing the official name. The use of the shorthand term and the official name for each product line is trademark protected under the overarching trademark declaration and protection request published on the radegenbio.com webpage.

θ.synth.clonal.construct (clonal constructs) - Clonal synthetic genetic constructs composed of virtually any sequence that includes a synthetic vector backbone. Synthetic DNA can either be defined as a novel sequence that never before existed in nature or DNA with a natural sequence that is made by coupling nucleotide by nucleotide. In both cases if the DNA is synthetic, then it must be synthesized in a stepwise fashion. When Radegen Bio synthesizes a plasmid, the entire plasmid, including the backbone is a synthetic piece of DNA. Rest assured that one of the strands of your plasmid is not from the ice age, and it’s age will not compromise your experiential integrity since our DNA is build block by block. With all sincerity, it is easier for Radegen Bio to build DNA this way! Oh, by the way. We do not charge a dime for the vector backbone and this product line will be at a competitive price point to non-clonal competitors. If you have a custom plasmid that is not part of our plasmid suite, cost of the plasmid will be incurred the first time and after your vector backbone synthetic parts will be saved for future orders. No more sending in a plasmid prep to a synthetic DNA provider, a process that can last months.

1. Use as building blocks for building larger synthetic constructs using traditional restriction enzyme/ligase-based approaches, Gibson assembly, Golden Gate Assembly and Gateway.
2. Use for developing a custom RNA or protein expression cassette.
3. Vector backbones amenable for Golden Gate Assembly, Gibson Assembly and Gateway Assembly are available and developed from peer reviewed academic sources. It is our goal to develop a nuclease using a Cas9 enzyme that produces blunted ends. This facilitates downstream Gibson assembly. A Cas based approached, is desired since Cas is just a fancy nuclease and has a large enough recognition sequence so one enzyme can be used for any customer sequence and Radegen Bio never needs to ask you to edit your sequence. Keep in mind that development literally entails designing a multiple cloning site with a palindromic adjacent motif sequence and a 25 bp recognition sequence for Cas9 binding. Cas9 is already a nuclease, a protein that cleaves DNA so development is not necessary on that regard. We are also in the works of developing a nuclease with an expanded nuclease that will revolutionize the enzyme industry since only one enzyme is needed to for producing all 256 4bp overhangs. This approach would is only possible using synthetic DNA. The following citation describes the

development of a new enzyme with a 18 bp recognition sequence (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2685105/). Why this enzyme was developed in 2009 is unclear since it’s recognition site is more laborious to generate and provides only the advantage of rarity beyond the point required for cloning. A 18 bp recognition site that provides 4bp overhangs would be the ultimate tool for type IIS based assembly of synthetic DNA from a manufacture’s perspective since a sequence would cut DNA once every 104,976 bp. Usually 6 bp is more than enough most of the times and 7 bp sequence hardly ever requires the introduction of a cloning scar.

4. Sanger Sequencing is used as the primary sequencing QC standard since it is the benchmark for sequencing clonal DNA material.

II. **ɵ**.CNTL.qPCR - Synthetic qPCR positive controls are DNA template sequences cloned in a vector and used as positive controls in qPCR applications. ⊖.CNTL.qPCR (qPCR controls) can be prepared with up to 6 target templates (150bp), will be available as single preparations with a goal of providing 15 µg of pladmid DNA yield. Radegen Bio plans on using silica column technology for DNA purification allowing for easier preparation of endotoxin free DNA. Our basic wash protocol produces preps with < 0.1 units of endotoxin per preparation. Certification will be available at an additional cost. Amplification templates are verified by Sanger Sequencing and UV spec IV.

III. **ɵ**.rollingcircle.cellfree.BAC – A bacterial cell free lysate is under development using the soil dwelling species, *Pseudomonas putida*. *Pseudomonas putida* is an excellent organism for protein expression since output is several times higher than that of *E. coli* based expression. This cell of bacteria will be engineered to encode for the factors required for rolling circle DNA amplification. Addition of only a few copies of a plasmid to a protein synthesis reaction provides the reaction template that exponentially grows providing RNA polymerase with plenty of material for transcription and translation. Ribosomes and factors associated with protein synthesis will also be overexpressed. These combined approaches, using a unorthodox protein expression species, that is engineered to expressed rolling circle replication machinery, engineered to over express protein expression factors, and lysates prepared from protoplast that lack in lps are Radegen Bio’s rational design approach that combines methods from the past 70 years of molecular biology research to produce high quality molecular solutions full of helpful features that make your life easier.

V. **ɵ**.rollingcircle.cellfree.EUK – A eukaryotic cell free lysate from a yeast strain is under development and in the conceptualization process. The same framework as the bacterial system will be produced.

VI. **ɵ.Pfu-S** – A PCR cell lysate master-mix using *P. putida* and a synthetic promiscuous dNTP synthase. **ɵ.Pfu-S** is overexpressed in *P. putida* along with the dNTP synthase. PCR using anything else after using such a simple approach. Microbiologist use colony PCR everyday where a single colony is dropped into PBS and primers to screen for a intended recombineering event. Most PCR application, including an under development Sybergreen lysate master mix can benefit from this method since the factors that make DNA replication in vivo are in situ an assist polymerase. This mechanism is not reported but I highly doubt that ribosomes mediate the process.

VII. ɵ.rollingcircle.cellfree.plasmid – A cell lysate for cell free plasmid propagation based on the ɵ.rollingcircle.cellfree.PRO system. The system lacks in protein synthesis factors but keeps the rolling circle amplification factors. This method is much more simple than the oriC system since it does not require as many proteins to achive the same process.

VIII. ɵ.cellfree.plasmid.prep - A plasmid prep produced in a cell free system from a theoretical single plasmid copy introduced into a plasmid propagation reaction by serial dilution. ϴ.cellfree.plasmid.prep (cell free plasmids) can be ordered either in a Radegen plasmid or as a wholly synthetic plasmid. Depending on DNA assembly complexity, cell free plasmid preps can be delivered within 48 buisness hours. Plasmid preps are available as single preperations with 1 µg of purified plasmid. QC is determined by Sanger Sequencing and UV Spec.

VII. ɵ.minicell.drives - is a nucleic acid based binary data storage system where plasmids containing binary information converted into a nucleic acid sequences are stored in non-replicating minicells. This system takes advantage of all the benefits provided in a single cell, including pre-storage amplification to help keep your data secure. Since we use only high fidelity, non-pool based synthesis, and since there is a lack of repetitive elements in a vector backbone that is being sequenced, NGS sequencing will be conducted. NGS will also be conducted on libraries to provide diversity QC data.

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